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**Intra-specific genetic diversity and composition modify species-level
diversity-productivity relationships**

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Tables of Supporting Information.

Summary

- Biodiversity regulates ecosystem functions such as productivity, and experimental studies of species mixtures have revealed selection and complementarity effects driving these responses. However, impacts of intra-specific genotypic diversity in these studies are unknown, despite it forming a substantial part of the biodiversity.
- In a greenhouse experiment we constructed plant communities with different levels of barley (*Hordeum vulgare*) genotype and weed species diversity and assessed their relative biodiversity effects through additive partitioning into selection and complementarity effects.
- Barley genotype diversity had weak positive effects on aboveground biomass through complementarity effects, whereas weed species diversity increased biomass predominantly through selection effects. When combined, increasing genotype diversity of barley tended to dilute the selection effect of weeds.
- We interpret these different effects of barley genotype and weed species diversity as the consequence of small versus large trait variation associated with intra-specific barley diversity and inter-specific weed diversity respectively. The different effects of intra- vs. inter-specific diversity highlight the underestimated and overlooked role of genetic diversity for ecosystem functioning.

Keywords: barley genotypes, complementarity effects, diversity-productivity relationship, genetic diversity, *Hordeum vulgare*, net biodiversity effects, sampling effects, species diversity, weeds

Introduction

Understanding biodiversity's role in regulating key ecosystem functions such as productivity is a major challenge for ecology. Diverse communities can utilise resources effectively and extensively, and can be more productive, resulting in a positive relationship between producer (e.g. vascular plant) diversity and productivity (e.g. Tilman *et al.*, 1997; Hector *et al.*, 1999; Hooper *et al.*, 2005; Cardinale *et al.*, 2011). Positive biodiversity-productivity relationships are thought to be generated by two mechanisms: (1) sampling or selection effects, (2) complementarity effects (Loreau & Hector, 2001). Selection effects involve an increased probability of more diverse species mixtures harbouring high-yielding species that increase productivity and simultaneously dominate the mixture (Aarssen, 1997; Huston,

1997; Tilman *et al.*, 1997). The productive species' dominance will lead to over-yielding, but never to transgressive over-yielding: the mixture will perform better than the average monoculture, but will not outperform the best monoculture. In contrast, complementarity effects from niche differentiation or facilitation lead to enhanced performance of species in mixtures and increased net resource use (Trenbath, 1974), and potentially transgressive over-yielding (Loreau, 2004).

As well as inter-specific diversity, intra-specific diversity may be important for the regulation of productivity (Reusch *et al.*, 2005; Crutsinger *et al.*, 2006; Hughes *et al.*, 2008; Fridley & Grime, 2010; Kotowska *et al.*, 2010; Cardinale *et al.*, 2011; Cook-Patton *et al.*, 2011; Crawford & Rudgers, 2012). Empirical and theoretical evidence also indicate the regulatory effects of intra-specific genetic diversity on species diversity (Booth & Grime, 2003; Vellend & Gerber, 2005; Whitlock *et al.*, 2007; Fridley & Grime, 2011; Gibson *et al.*, 2012): genetic diversity in populations has direct consequences for community species diversity through competition or niche differentiation. Consequently, besides direct effects of intra-specific diversity on productivity, intra-specific diversity could also indirectly affect the diversity-productivity relationship by influencing species diversity.

Key policy documents frequently promote the conservation of genetic diversity (e.g. United Nations Convention on Biological Diversity, 2010), but we have limited understanding of the relative role of genetic diversity in diversity-function relationships (Hughes *et al.*, 2008). We are not aware of any study that quantifies selection *vs.* complementarity effects in experiments that combine both genotype and species mixtures, and that compares the relative effects of intra- and inter-specific diversity on productivity. This is likely due in part to the difficulty of regulating and keeping track of intra-specific diversity in mixtures. However, annual crop systems provide an excellent resource for exploring the role of genetic diversity. Although intensive agricultural crops are normally genetically uniform (i.e. comprising a single cultivar), genotype mixtures of multiple cultivars have been receiving more attention (Newton *et al.*, 2009). Indeed, the range of available elite cultivars provides varied and genetically characterised material from which to construct mixtures with controlled levels of genotypic variation.

Diversity impacts on ecosystem functions may not be driven by species or genotype number *per se*. Recent interest has focussed on functional diversity and its impact on ecosystem processes such as nutrient turnover and system productivity (Hillebrand & Matthiessen, 2009; Newton *et al.*, 2009). Critically, functional diversity may mediate the effects of intra- and inter-specific diversity on ecosystem functions (Díaz *et al.*, 2007),

including productivity (Cadotte *et al.*, 2009; Flynn *et al.*, 2011; Roscher *et al.*, 2012, 2013). Functional diversity can regulate resource exploitation and niche complementarity, and so the impacts of genotypic or species diversity might be determined by their relative influence on functional traits. Species diversity could contribute more than intra-specific genotype diversity to variation in functional traits, and therefore ecosystem functions (Auger & Shipley, 2013).

We hypothesized that increasing genotype or species diversity would lead to positive net biodiversity effects on productivity, but that the effect of species richness would be stronger than the effect of intra-specific genotype richness, in line with the expected higher trait variation associated with species diversity compared to genotype diversity. Besides direct effects of genotype and species diversity on productivity, we hypothesized that genotype richness would modulate the effects of species richness on community productivity.

To test these hypotheses, we constructed communities of annual plants and manipulated both intra-specific genotype (barley cultivars) diversity and (weed) species diversity. To assess whether net biodiversity effects resulted from selection or complementarity effects, and how these were influenced by intra- vs. inter-specific diversity, we used additive partitioning *sensu* Loreau & Hector (2001).

Material and Methods

We constructed mesocosm communities in a greenhouse at the James Hutton Institute, Aberdeen (UK). The communities had varying weed species and barley cultivar diversity and were sown in wooden, soil-filled boxes (0.7 x 0.7 x 0.3 m; surface area 0.49 m²), lined with plastic and filled with homogenised loam topsoil over approx. 5 cm of c. 20 mm granite gravel. Mesocosms were kept under natural daylight and received no fertiliser, being watered uniformly with 1-2 l water per mesocosm 2-3 times per week. Watering volume and frequency increased as the plants grew, and depended also on the weather. Air temperature and relative humidity in the greenhouse varied temporally between 5 and 25 °C and 40 and 100% respectively.

All plants were planted between 6 and 14 June 2012, except for Oxbridge barley (19 June) and *Euphorbia helioscopia* (18 July) which had delayed planting dates due to low seed viability. Before planting, all seeds were soaked overnight in 1 µM gibberellic acid (GA; Sigma-Aldrich Chemie GmbH, Steinheim, Germany); after GA treatment, seeds were spread onto a layer of commercial seedling compost and maintained at 5 °C in the dark for two days

to synchronise germination. Germinated weeds were transferred to the greenhouse under ambient temperature and daylight conditions for three additional weeks. Barley seedlings were grown at 20/18 °C with a 12-hour day/night regime for only one week prior to transplanting. To ensure standardised arrangement of plants within mesocosms, seedlings were planted in a grid pattern defined by a square wooden frame, with stretched strings dividing the mesocosm surface into 64 equal-sized squares whose centres marked the planting points. For each mesocosm, 60 seedlings were transplanted individually into the square units, with equal proportions of each barley cultivar or weed species in mixtures (detailed below). This ensured a planting density similar to barley crop fields. Plants of each cultivar or species were randomly allocated to grid squares, leaving four empty squares per mesocosm. In mesocosms with all five barley cultivars, different cultivars were identified using colour-coded wire rings secured loosely around the stem base of each barley plant.

Mesocosms were monitored twice weekly for plant survival, health and development; here we report final biomass data. To prevent lodging the tops of all barley plants were brushed gently with a wooden stick five times a week for 10 weeks after planting (a technique commonly used when growing cereals in greenhouse conditions). Biomass of volunteer weeds was extremely low (final harvest mean \pm SE = 0.15 ± 0.02 g dry mass mesocosm⁻¹) and is unlikely to have influenced the much greater biomass (on average 584 times larger) of the barley and experimental weed species considered here. To limit heavy infestations of powdery mildew and aphids, plants were treated with a fungicide (1 ml/l; ALTO 100 SL, Syngenta Crop Protection Inc., Greensboro, USA) and soap solution (10 ml/l; SAVONA, Koppert B.V., AD Berkel en Rodenrijs, NL) at 30 and 80 days to ensure consistent survival of plants across mesocosms and treatments. All plants in each mesocosm were harvested between 100 and 104 days after planting, except Oxbridge and *E. helioscopia* (harvested at 91-95 and 62-66 days, respectively). For each mesocosm, aboveground plant material was removed at the stem base, and separated into species and barley cultivars. Dry mass of each species and cultivar was recorded after at least 72 h in an 80 °C oven.

Diversity treatments

Intra-specific genotype diversity was manipulated using combinations of five barley (*Hordeum vulgare* L.) cultivars (Optic, Oxbridge, Sebastian, Tipple, and Westminster, hereafter called “genotypes”) showing a genetic similarity between 61-68% based on single nucleotide DNA polymorphisms (see Notes S1 in Supporting Information) and species diversity with combinations of five annual weeds common in barley crops [*Capsella bursa-*

pastoris (L.) Medik. (Brassicaceae), *Euphorbia helioscopia* L. (Euphorbiaceae), *Poa annua* L. (Poaceae), *Senecio vulgaris* L. (Asteraceae), and *Stellaria media* (L.) Vill. (Caryophyllaceae)]. It is important to note that the weed seeds - obtained from a commercial supplier (Herbiseed, Twyford, UK) - included an unknown number of genotypes. In this experiment we compared, therefore, intra-specific genotype diversity (of barley) with species diversity (of weeds) where the latter also includes an unknown element of intra-specific genotype diversity (as is the case in similar species diversity experiments). Consequently, we interpret the differences in the effects of varying barley and weed diversity as predominantly a consequence of differences in inter-specific diversity, whereas responses in the barley mixture treatments alone represent intra-specific diversity effects.

Barley genotypes were chosen to include contrasting weed resistance abilities, based on experimental field plot data (A.C. Newton & S. Hoad, pers. comm.). To manipulate species diversity we followed a conservative approach by not including N-fixing weeds because their impact can mask diversity effects (Cadotte *et al.*, 2009). Genotypes and species were grown in monoculture (one [B]arley or one [W]eed – 10 treatments; all 60 plants in each mesocosm being the same genotype or weed species), and mixtures of either all five genotypes (5B – 1 treatment, 12 plants per barley genotype), all five weed species (5W – 1 treatment, 12 plants per weed species), one barley genotype and five weed species (1B5W – 5 treatments, 10 plants per genotype or weed species), and five barley genotypes & five weed species (5B5W – 1 treatment, 6 plants per genotype or weed species). Therefore, barley genotype and weed species richness both varied between zero, one and five (see Table S1). Four replicates of each treatment and 18 different species \times genotype combinations resulted in 72 mesocosms in total. Importantly, due to space constraints, this design is not fully-factorial: no mixtures contained only a single weed species (i.e. there are no 1B1W and 5B1W combinations). Hereafter we refer to combinations with more than one barley genotype and/or weed species as mixtures, and those with one barley genotype and no weeds, or one weed species and no barley, as monocultures.

Trait measurements

In order to assess trait variation of the mixture components (i.e., the barley cultivars and weed species) we randomly selected five individuals of each weed species or barley cultivar within each of the four 5B5W mesocosms and measured plant height, plant width, biomass, leaf area, specific leaf area (SLA), leaf dry matter content (LDMC) and relative water content (RWC), whereas leaf carbon and leaf nitrogen concentrations were determined for all

individuals of the same cultivar or species and mesocosm pooled. These traits were expected to reflect various aspects of the niche of each cultivar and species (*sensu* Violle & Jiang, 2009), such as growth rate, competitive ability and resource use (light, water and nutrients). Trait measurements except biomass were performed 56-63 days after planting the first seedlings, except for Oxbridge (51-58 days) and *E. helioscopia* (21-28 days). Biomass was determined at final harvest between 100 and 104 days after planting, except Oxbridge and *E. helioscopia* (harvested at 91-95 and 62-66 days, respectively).

For most traits we followed the protocol of Cornelissen *et al.* (2003) for trait measurements. Plant height (cm) was measured as the vertical distance between the uppermost tip of photosynthetic tissue and the ground level. Plant width (cm) was measured as the horizontal distance between the two outermost tips of photosynthetic tissue. For plant biomass (g) aboveground plant material of each individual was removed at the stem base, and dry mass recorded after at least 72 h in an 80 °C oven. For leaf area (mm²), SLA, LDMC and RWC we carefully removed a fully developed and healthy leaf of each plant individual, determined fresh mass at field turgor, fully rehydrated the leaf overnight and measured fresh mass at full turgor and leaf area. After drying the leaf at 80 °C for 48 h we determined dry mass. SLA was then calculated as the ratio between leaf area of the fresh leaf and leaf dry mass (mm² mg⁻¹), LDMC as the ratio between leaf dry mass and fully rehydrated fresh mass (mg g⁻¹), and RWC as the ratio between leaf water content in the greenhouse and water content after rehydration at full turgor (%). Leaf water content in the greenhouse was calculated as the difference between field fresh mass and dry mass, and water content at full turgor was calculated as the difference between saturated mass and dry mass. For leaf C and N concentrations (% of dry mass) the dried leaves of each cultivar or species of each mesocosm were pooled, ball milled to fine powder and total C and N contents of a 2 mg subsample were determined using an Exeter Analytical CE440 Elemental Analyzer (EAI, Coventry, UK). For further analysis we used mean trait values per cultivar or species and mesocosm.

Additive partitioning of biodiversity effects

Following Loreau & Hector (2001), the net biodiversity effect was determined as the observed absolute performance (i.e. aboveground biomass [g] per mesocosm) of mixtures compared to their expected performance based on the relevant monocultures. Net biodiversity effects are the sum of two groups of underlying effects with different causes – the selection effect and the complementarity effect.

Selection effects are quantified as the covariance between the biomass of a species or genotype in monoculture and the change in relative yield of each species or genotype grown in mixture (see Notes S2). Positive selection effects indicate dominance in the mixture by species or genotypes that perform best in monoculture. However, they do not lead to increased net performance of the mixture above that expected from the best performing monoculture. Negative selection effects occur if species that perform poorly in monoculture dominate a mixture (Loreau & Hector, 2001).

Complementarity effects are indicated by changes in the performance of a species or genotype in mixture that are not compensated by similar-scale but opposing changes in other mixture components. They quantify the change in mixture yield relative to that expected from the monocultures (see Notes S2). As with selection effects, complementarity effects can be positive or negative (Loreau & Hector, 2001). Increasing performance of one component in the mixture without a balancing decrease in performance in another component results in a positive complementarity effect, and enhanced net performance of the mixture based on expectations from weighted monoculture means (Turnbull *et al.*, 2013).

Statistical Analyses

A linear mixed-effects model was applied to test for differences in aboveground biomass per mesocosm between monocultures and mixtures, including the treatments as a random variable.

For each mixture significant negative or positive selection, complementarity and net biodiversity effects (i.e. significant deviations of these scores away from zero) were assessed using two-tailed Student's one-sample *t*-tests. Comparisons of biodiversity effects between 5B, 5W and 5B5W mixtures were conducted using two-tailed Welch two-sample *t*-tests.

Linear mixed-effects models were applied to test relationships between genotype and species richness and selection, complementarity and net biodiversity effects on aboveground mixture biomass. Barley genotype composition was included as a random effect consisting of seven levels (none, Optic, Oxbridge, Sebastian, Tipple, Westminster, mixture). Fixed effects of 'genotype richness' and 'species richness' were assessed using type-II analyses of variance and Wald χ^2 -test statistics. Fixed effect importance was calculated as the product of the slope (i.e. factor coefficient) and maximum range of the factor covered by the experiment (Connolly *et al.*, 2011). The lack of a fully factorial design prevented exploration of interactive effects of genotype and species richness. The random effect 'genotype composition' was assessed using likelihood ratio tests comparing the models with and

without this random effect (Hector *et al.*, 2011). Weed species composition effects could not be assessed as either none or five weed species were present in mixtures.

We performed a principal component analysis of the nine traits monitored for the five barley cultivars and the five weed species grown in the 5B5W mixture (Table S2). Prior to analysis trait values were scaled to unit variance and zero centred because traits were in different units and had different variances. We also applied a Varimax rotation to reduce the number and multi-collinearity of variables.

All statistical analyses were performed with R version 2.15.3 (R Core Team, 2013) and *nlme* (Pinheiro *et al.*, 2013).

Results

Diversity effects on aboveground biomass

Overall, mixtures (110.3 ± 5.4 g mesocosm⁻¹) had 41% higher average biomass than monocultures (78.2 ± 8.2 g mesocosm⁻¹; $F_{(1,70)} = 9.28$, $P = 0.003$). However, no mixture produced as much biomass as the most productive monoculture *Stellaria* (Fig. 1).

Partitioning of biodiversity effects

Barley genotype diversity was associated with a marginally significant net biodiversity effect (Fig. 2), with a 7.9% increase in biomass in the 5B mixture compared to that predicted from monocultures. Although neither selection nor complementarity effects were significant, this tendency towards a positive net biodiversity effect appears to be driven by weak positive complementarity effects (Fig. 2).

Weed species diversity was associated with a significant positive net biodiversity effect driven by a strong selection effect (Fig. 2), resulting in a 68% biomass increase in the 5W mixture compared to that expected from monocultures. This selection effect was caused by dominance of *Stellaria* (+184%) and *Senecio* (+23%) and declines in *Euphorbia* (-52%), *Poa* (-73%) and *Capsella* (-80%).

For 1B5W mixtures overall (i.e. pooling data from across mixtures containing different barley genotypes) we found a highly significant net biodiversity effect ($t = 5.94$, d.f. = 19, $P < 0.001$). Again, this was driven by a strong selection effect ($t = 9.82$, d.f. = 19, $P < 0.001$), with no significant complementarity effect ($t = 0.59$, d.f. = 19, $P = 0.56$). Small differences occurred between 1B5W mixture types, indicating that weed species diversity effects depended on the barley genotype present (Fig. 2, and see Table S2). Nevertheless, in nearly

all 1B5W mixtures there was a significant or marginally significant net biodiversity effect driven by a significant selection effect (Fig. 2). The exception to this was the mixture containing the genotype Optic, which showed only a marginally significant selection effect. Overall, the selection effect was driven by increased biomass of *Stellaria* ($176 \pm 19\%$) and barley ($27 \pm 7\%$) at the expense of *Senecio* ($-3 \pm 12\%$), *Euphorbia* ($-42 \pm 8\%$), *Capsella* ($-73 \pm 4\%$), and *Poa* ($-75 \pm 4\%$). Of the barley genotypes, highest relative mass gain in 1B5W mixtures compared to monocultures was observed for Sebastian ($+54\%$), followed by Oxbridge ($+28\%$), Tipple ($+21\%$), Westminster ($+19\%$), and Optic ($+12\%$).

The 5B5W mixture showed a significant positive selection effect and a marginally significant positive net biodiversity effect (Fig. 2). The selection effect was due to dominance by *Stellaria* ($+211\%$) and Sebastian ($+20\%$) at the expense of Optic (-4%), Oxbridge (-17%), Tipple (-18%), Westminster (-27%), *Senecio* (-33%), *Euphorbia* (-73%), *Poa* (-77%), and *Capsella* (-86%). The only marginally significant net biodiversity effect may have resulted from the negative, but non-significant, complementarity effect counteracting the positive selection effect.

Effects of changes in barley genotype or weed species richness on biodiversity effects

Although net biodiversity effects and their components (i.e. selection and complementarity effects) for individual barley genotype mixture treatments were not or only weakly significant (as reported above; Fig. 2), combining data from across mixture treatments showed that net biodiversity effects in mixtures tended to decrease with increasing barley genotype richness (Fig. 3a, Table 1), with smallest net biodiversity effects in 5B mixtures driven by declining trends in both selection (Fig. 3b) and complementarity effects (Fig. 3c). Barley genotype diversity impacts on selection and complementarity effects were of similar importance (Table 1).

Net biodiversity effects did not change with changes in weed species richness from 0 to 5 (Fig. 3d, Table 1), but this hides underlying but counteracting changes in selection and complementarity effects. Selection effects increased with weed species richness (Fig. 3e), but complementarity effects tended to decrease (Fig. 3f, Table 1). Species diversity was more important for selection than complementarity effects. Compared to the effects of barley genotype diversity, weed species diversity was more important for the selection effect and of similar importance for the complementarity effect (Table 1).

The net biodiversity effect ($t = 4.03$, d.f. = 5.54, $P = 0.008$) and the selection effect ($t = 8.87$, d.f. = 3.02, $P = 0.003$), but not the complementarity effect ($t = -0.80$, d.f. = 5.98, $P =$

0.45) were both significantly greater for 5W compared to 5B mixtures, indicating stronger biodiversity effects by weed species than barley genotypes through stronger selection effects. The 5B5W mixture had significantly larger selection effects compared to the 5B mixture ($t = 3.28$, d.f. = 3.01, $P = 0.046$), and marginally significantly smaller selection effects than the 5W mixture ($t = -2.17$, d.f. = 5.21, $P = 0.080$). Net biodiversity and complementarity effects did not differ significantly between 5B or 5W and 5B5W mixtures. This illustrates overall dilution by 5B mixtures of selection effects, for example when combined with five weed species in the 5B5W combination.

The random effect ‘genotype composition’ significantly influenced the response of the net biodiversity, selection, and complementarity effects to changes in species and genotype diversity (Table 1), indicating that these effects differed between barley genotypes (Fig. 2).

Trait variation in barley and weed mixtures

The trait values summarised in Table 2 indicate lower trait variation among the five barley genotypes compared to the five weed species. This was confirmed with the species scores plotted on the first to third principal components (Fig. 4), which showed substantially more trait variation among weed species than among barley cultivars. The first principal component (explaining 27% of the trait variation among species and cultivars) had high positive variable loadings of plant height, leaf area, LDMC and leaf C and high negative loadings of SLA and leaf N (Table S2). The second principal component (23%) had high positive loadings of leaf N and high negative loadings of plant width and biomass. The third principal component (17%) had a high positive variable loading of RWC.

Discussion

In our crop-weed system we observed over-yielding of mixtures but no transgressive over-yielding. A positive net biodiversity effect was observed for the weed species mixtures (5W), but became weaker when five barley genotypes were included (5B5W). The higher net biodiversity effect for weed species mixtures compared to barley genotype mixtures, or combined barley and weed mixtures, was attributable to large selection effects in weed species mixtures. These were absent in exclusively intra-specific barley genotype mixtures (5B) and much reduced under high barley genotype richness in weed species mixtures (i.e. the 5B5W treatment). Net biodiversity effects in intra-specific barley genotype mixtures operated mainly through complementarity effects, which were generally weak and influenced to a similar extent also by weed species diversity. Overall, increases in both barley genotype

and weed species diversity led to weak complementarity effects, whereas increased species diversity led to strong positive selection effects; intra-specific barley genotype diversity was crucial in modulating the direction and strength of the weed species mixtures' selection effects.

Effects of intra- and inter-specific diversity

Barley genotype (5B) mixtures showed relatively weak positive net biodiversity effects due to weak positive complementarity effects; no single genotype dominated the barley mixtures. Weak effects of intra-specific genotype diversity on productivity have been found previously in agricultural and natural vegetation systems (Booth & Grime, 2003; Newton & Guy, 2009; Fridley & Grime, 2011). In these studies, detection of net biodiversity effects was perhaps limited by the relatively small variability in performance encompassed by genotypic diversity. The same may have been true here: total aboveground biomass of the best performing monoculture genotype, Westminster, was 1.5 times that of the worst performing, Oxbridge (Fig. 1). Studies finding substantial effects of intra-specific diversity used more variable genotypes, with monoculture performance varying between genotypes by a factor exceeding two (Reusch *et al.*, 2005; Crutsinger *et al.*, 2006; Kotowska *et al.*, 2010).

The variability in potential performance encompassed by the weed species (5W) mixtures was much higher, with monoculture performance varying by a factor of 17 between *Stellaria* and *Euphorbia*. We found a strong positive net biodiversity effect: weed species mixtures produced 78% greater biomass than expected from monoculture yields. This scale of effect is within the average range of species richness effects on productivity found in other studies in natural systems (Cardinale *et al.*, 2007). The positive biodiversity effect in weed species mixtures can be attributed almost exclusively to a selection effect; the most productive weed species *Stellaria* dominated the mixture, compensating for productivity declines in less productive species.

The proposal that lower trait variability is associated with intra-specific genotype diversity compared to species diversity is supported by examination of the trait space occupied by barley genotypes and weed species (Fig. 4). They illustrate the much larger trait space volume occupied by the weed species compared to the barley genotypes in 5B5W mixtures. This analysis demonstrates *Stellaria*'s distinct combination of traits (Fig. 4), including rapid growth and large biomass (Table 2), making *Stellaria* an obvious potential driver of significant positive selection effects. It also demonstrates the considerable trait variation between the five weed species. We selected five phylogenetically distantly-related species

and this may have enhanced functional trait variability. Previous studies showed that phylogenetic distance and corresponding trait differences in species mixtures are very strong predictors of species diversity effects on productivity (Cadotte *et al.*, 2009; Flynn *et al.*, 2011). Our results (comparing the functional variability associated with either species or intra-specific genotype diversity) confirm these previous findings. Strong selection effects are likely in species mixtures where one species shows extreme trait values (Loreau, 2000), particularly in short-term studies of rapidly-growing annual plants (Cardinale *et al.*, 2007; Fargione *et al.*, 2007; Jiang *et al.*, 2009).

Interaction effects between intra- and inter-specific diversity

Positive net biodiversity or selection effects occurred in all weed species mixtures irrespective of the presence and diversity of barley genotypes (i.e. *Stellaria* dominated in 5W, 1B5W and 5B5W mixtures). However, intra-specific diversity of barley influenced this in two ways.

First, the direction and strength of the net biodiversity, selection, and complementarity effects were influenced by barley genotype identity in 1B5W mixtures: different barley genotypes created different dominance patterns (see Table S3). Productivity increases in *Stellaria* and barley (irrespective of genotype) in mixtures generally compensated for declines in productivity by other weed species, but relative mass gains and losses of all mixture components varied considerably. We interpret this as variation in interaction intensities depending on the barley genotype present. Genotype-dependency of inter-specific competition has been widely reported (Taylor & Aarssen, 1990; Lankau & Strauss, 2008; Fridley & Grime, 2010), particularly for spring barley (Hansen *et al.*, 2008). Fridley *et al.* (2007) demonstrated that genotype-specific effects can alter dominance patterns and compensatory growth of co-existing species. Differences in barley genotype competitive ability might therefore drive variation in compensatory growth patterns among the 1B5W treatments, and the corresponding variation in selection and net biodiversity effects.

Second, although increasing barley genotypic diversity did not in itself lead to strong biodiversity effects on community productivity, overall increases in barley genotypic richness tended to dilute selection effects. As barley genotypic diversity increased from 0 to 1 to 5, selection effects - driven mainly by weed species diversity - tended to decline (Fig. 3b). Previous studies have indicated that intra-specific genotype mixtures can alter biomass compensation patterns of co-occurring species. Crawford & Rudgers (2012) showed that when genetic diversity of the dominant *Ammophila breviligulata* in a dune system was low,

the relationship between species richness and community-level productivity was negative or neutral. However, under high *Ammophila* genetic diversity the relationship became positive. In contrast, increasing barley genotype diversity in our communities had negative effects on relationships between species richness and productivity. We hypothesise that the differences in responses may be related to the dominance of the particular species within which genetic diversity is changing. High genotypic diversity of *dominant* species (e.g. *A. breviligulata*) might allow greater niche space occupancy, reduce intra-specific competition, and increase dominance. Therefore, increasing genetic diversity within dominants enhances the selection effect in species mixtures. In contrast, high genotypic diversity of *subordinate* species (e.g. barley) might reduce community level productivity by reducing the dominance of the most productive species. Therefore, the enhanced overall competitive impact of barley due to increasing genotypic diversity from 0 to 1 to 5 increasingly constrains the selection effect. In summary, enhanced intra-specific diversity promotes positive complementarity effects through niche differentiation, in turn increasing or reducing selection effects in species mixtures depending on the dominance of the species within which genetic diversity increases. Few studies are available to test this tentative mechanism, but it seems plausible and represents an interesting future research target.

Enhanced weed species richness had the opposite effect to barley genotype richness, significantly increasing selection effects (Fig. 3e). This contrast is crude, as weed species richness was 0 or 5 (meaning that weed species were either all absent or all present in mixtures), but demonstrates the limited potential for selection effects when the mixture contains only intra-specific diversity (5B mixtures). Both barley genotype and weed species richness were associated with trends toward declining complementarity effects (Fig. 3c,f). Net biodiversity effects are the sum of selection and complementarity effects. The consequence is that with increasing barley genotype richness there is a trend toward a decline in net biodiversity effects (Fig. 3a), whereas with increasing weed species richness there is no change in net biodiversity effects as the positive effects of weed species richness on selection effects, and its negative impact on complementarity effects, cancel each other out.

In conclusion, even though intra-specific genotype diversity had weak direct effects on community-level productivity, it had significant indirect effects by modulating the species diversity-productivity relationship. This indirect effect mainly causes a change in the dominance patterns of the co-existing species, observable as a change in the selection effect of species diversity on productivity.

Trait-based approach to biodiversity effects

The net effect of inter-specific diversity on productivity was about eight times higher than that of intra-specific genotype diversity, the main difference being strong, positive selection effects in species mixtures. Intra-specific diversity had weak effects on productivity, acting mainly through complementarity effects (see also Cook-Patton *et al.*, 2011). Here, we propose a functional trait-based framework that explains different effects of intra- and inter-specific diversity, and between-study differences in the magnitude of biodiversity effects, as well as temporal changes in the size of biodiversity effects. Trait-based approaches successfully explain biodiversity effects in species mixtures (Loreau, 2000; Cadotte *et al.*, 2009; Flynn *et al.*, 2011; Roscher *et al.*, 2012, 2013). Our framework extends to the effects of intra-specific diversity on productivity, and we propose that genotypes and species may have complementary effects on productivity through complementarity and selection effects respectively.

Intra-specific trait variability is generally smaller than inter-specific variation (Fig. 4; Auger & Shipley, 2013). Limited intra-specific trait variation likely excludes the occurrence of extreme trait values needed for selection effects (Loreau, 2000), but is sufficient for niche differentiation (Jung *et al.*, 2010) and hence complementarity effects. Complementarity effect size may then depend on trait variation, with more niche differentiation if trait variation is relatively high (but still below a threshold for selection effects). Our five barley genotypes showed a relatively small trait range despite being selected for distinctive weed-competitive traits (Fig. 4). This may explain their weak complementarity effects.

As trait variation increases, for example switching from genotype to species mixtures, selection effects are more likely (Loreau, 2000). Our dominant species, *Stellaria*, has relatively unique traits (Fig. 4), appearing to promote its dominance in mixtures. This leads to compensatory effects on co-occurring species and ultimately a selection effect. The selection effect is therefore likely important for net biodiversity effects in multi-species communities. As with complementarity effects, the magnitude of selection effects will co-vary positively with trait variation (Cadotte *et al.*, 2009; Roscher *et al.*, 2012).

We propose, therefore, that the relatively small trait variation associated with intra-specific diversity is the main driving force behind complementarity effects (in both genotype mixtures and in species mixtures that include intra-specific genetic diversity), whereas the relatively large trait variation associated with inter-specific diversity leads to selection effects. As noted, we did not control for intra-specific genotypic diversity within our weed species, but analysis of the within-species trait variation indicates that intra-specific trait

variation is similar between barley and most of the weed species (Table S4). This confirms that the increased trait variation we observed in weed species mixtures as opposed to barley genotype mixtures predominantly results from enhanced inter- rather than intra-specific diversity.

Studies following biodiversity effects through time in species richness experiments with defined communities have indicated that selection effects are generally relatively constant (van Ruijven & Berendse, 2005; Cardinale *et al.*, 2007; Fargione *et al.*, 2007). If selection effects are driven mainly by between-species differences of species mixtures, this will be stable if species diversity is maintained. By contrast, the same studies demonstrate temporal increases in complementarity effects. As intra-specific genetic diversity and composition of these experimental assemblages is very likely to change over time due to genetic drift and selection (Nestmann *et al.*, 2011), we propose that these changes are responsible for temporal increases in complementarity effects in natural systems.

The weaker complementarity compared to selection effects in our study may therefore be partially due to its short duration preventing genetic diversification or drift in the experimental communities. This could also explain the absence of transgressive overyielding, a phenomenon thought to arise due to temporally-increasing complementarity effects (van Ruijven & Berendse, 2005; Cardinale *et al.*, 2007; Fargione *et al.*, 2007). Our experimental duration was close to the field situation for annual crops. Consequently, complementarity effects may be limited in annual crop mixtures and transgressive overyielding rare unless crop mixtures have rather high initial intra-specific diversity and rather high trait variation without extreme trait values (see Kiær *et al.*, 2012). To increase complementarity effects, the design of genotypic mixtures of single species annual crops might therefore focus particularly on promoting genotypic diversity, but without extreme trait values. In contrast, mixtures involving combinations of species with very distinct trait values - for example classic intercrops - may result in positive selection effects. Thus their productivity might be improved further by increasing genetic diversity in the dominant species. Consequently, intercrops of genotypes or species, or both combined, may increase yield over average monocultures and particularly help to increase yield stability over time, as species and genotype mixtures are less prone to environmental fluctuations than a corresponding monoculture (Booth & Grime, 2003; Kiær *et al.*, 2012; Finn *et al.*, 2013).

Conclusion

Combining intra-specific genotype mixtures and inter-specific species mixtures revealed that

intra-specific genotype diversity may affect productivity through complementarity effects, whereas between-species differences promote selection effects on productivity. We propose, using a trait-based framework, that limited trait variation associated with within-species genetic diversity is responsible for the complementarity effects in diversity-productivity studies. In contrast, wider trait variation associated with between-species diversity drives selection effects whereby some species dominate and suppress subordinate species. We also propose that the direction and strength of selection effects are likely driven by genetic and phenotypic variation within dominant versus subordinate species and their corresponding complementarity effects. Strong complementarity effects within dominant species will increase selection effects, and complementarity effects among subordinate species will reduce them. These findings indicate the crucial importance of intra-specific genetic diversity for ecosystem functioning and the necessity to account for genetic diversity in studies of biodiversity effects on ecosystem functions.

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- Supporting Information**
- Notes S1** Detailed description of barley cultivars.
- Notes S2** Additive partitioning of biodiversity effects.
- Table S1** Richness, composition and replication of the 18 different treatments with barley cultivars and weed species grown in monocultures and mixtures.
- Table S2** Variable loadings of traits on the first three principal components.
- Table S3** Mass changes of species in the IB5W mixtures.
- Table S4** Intra-specific trait variation of barley and the five weed species.

Fig. 1. Mean (\pm 1SE) aboveground biomass per mesocosm for monocultures (diamonds) and mixtures (circles). Monocultures were grown for each of five barley cultivars (open diamonds) and five weed species (filled diamonds), whereas mixtures either comprised all five barley cultivars (5B), all five weed species (5W), one barley cultivar (Op = Optic, Ox = Oxbridge, Se = Sebastian, Ti = Tipple, We = Westminster) and five weed species (+5W), or five barley cultivars and five weed species (5B5W). Dashed line = average aboveground biomass per mesocosm for monocultures; solid line = average aboveground biomass per mesocosm for mixtures. For each treatment $n = 4$.

Fig. 2. Net biodiversity, selection, and complementarity effects for the mixtures of barley genotypes and weed species. B = barley genotype (Op = Optic, Ox = Oxbridge, Se = Sebastian, Ti = Tipple, We = Westminster), W = weed species. Mixtures were either five barley genotypes (5B), five weed species (5W), one barley genotype combined with five weed species (barley cultivar +5W), and five barley genotypes combined with five weed species (5B5W). Values shown ($\text{g } 0.5 \text{ m}^{-2}$) are treatment means \pm 1SE. Differences of means from 0 were tested with Student's one sample t -test, with significance levels indicated as ** ($P < 0.01$), * ($P < 0.05$), † ($P < 0.1$), ns ($P > 0.1$).

Fig. 3. The partial effects of changing barley genotype richness (a-c) and weed species richness (d-f) in crop-weed mixtures on the net biodiversity effect (a,d), selection effect (b,e), and complementarity effect (c,f). See Table 1 for the corresponding statistical results. The model fit (solid line) and associated 95% confidence intervals (dashed lines) shows the change in the biodiversity effects ($\text{g } 0.5 \text{ m}^{-2}$) with changing genotype (green) and species richness (red). Symbols show the observed value of each mixture mesocosm. In panels (a) - (c) filled triangles and circles stand for mixture mesocosms with 0 and 5 weed species respectively. In panels (d) – (f) open triangles, squares and circles stand for mixture mesocosms with 0, 1 and 5 barley cultivars respectively. Symbols at number of genotypes or number of species equal to 5 were jittered to enhance visibility.

Fig. 4. Principal component analysis of nine traits (plant height, plant width, biomass, leaf area, specific leaf area, leaf dry matter content, relative water content, leaf carbon and leaf nitrogen concentration) of the five weed species (red) and five barley cultivars (green) in the four mesocosms consisting of the 5B5W mixture. Principal components 1, 2 and 3 explained 27%, 23% and 15% of the variance respectively. Weed species: Cb = *Capsella bursa-pastoris*, Eh = *Euphorbia helioscopia*, Pa = *Poa annua*, Sv = *Senecio vulgaris*, Sm = *Stellaria media*; barley cultivars: Opt = Optic, Oxb = Oxbridge, Seb = Sebastian, Tip = Tipple, Wes =

735 Westminster.

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Table 1. The effects of barley genotype and weed species richness (fixed effects) and barley genotype composition (i.e. the identity of the barley cultivar, random effect) on net biodiversity, selection and complementarity effects calculated from aboveground biomass. The table shows for each model variable the degrees of freedom (d.f.), χ^2 -value, *P*-value, factor coefficient (Coef) or likelihood ratio test score (logLikelihood) for fixed and random effects respectively, and estimate of importance for the fixed effects calculated as the division of the slope by the factor range (see Methods for details)

Effect variable	Model effects	d.f.	χ^2	<i>P</i> -value	Coef/ logLikelihood	Importance
Net biodiversity	Fixed - Genotype richness	1	3.31	0.069	-6.787	34
	Fixed - Species richness	1	0.01	0.921	0.408	2
	Random - Genotype composition	1	13.61	<0.001	-145.413	
Selection	Fixed - Genotype richness	1	2.88	0.090	-3.661	18
	Fixed - Species richness	1	5.68	0.017	5.697	28
	Random - Genotype composition	1	10.32	0.001	-129.525	
Complementarity	Fixed - Genotype richness	1	3.05	0.081	-3.464	17
	Fixed - Species richness	1	3.05	0.081	-3.840	19
	Random - Genotype composition	1	9.81	0.002	-127.074	

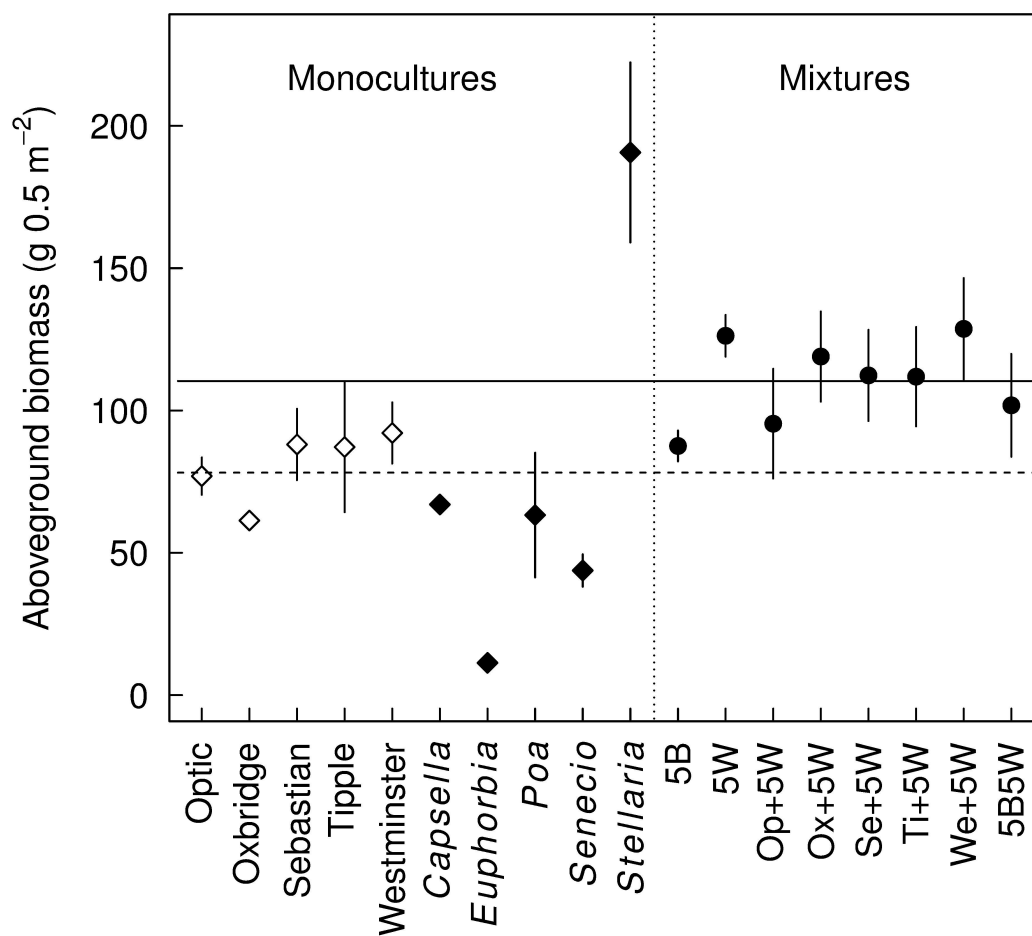
746 **Table 2.** Trait values (mean \pm 1SE) of nine traits measured for the five barley cultivars and five weed species in the 5B5W mixture mesocosms.
747 $n = 4$ (except *Poa* and *Euphorbia*, where $n = 2$ and $n = 3$ respectively due to mortality).

Cultivar / Species	Plant height (cm)	Plant width (cm)	Biomass (g)	Leaf area (mm ²)	SLA (mm ² mg ⁻¹)	LDMC (mg g ⁻¹)	RWC (%)	C (%)	N (%)
Optic	53.8 \pm 1.1	21.8 \pm 1.1	1.169 \pm 0.082	1770 \pm 77	42.9 \pm 0.8	161.0 \pm 5.0	90.6 \pm 1.7	41.9 \pm 0.3	3.75 \pm 0.12
Oxbridge	46.0 \pm 1.9	22.6 \pm 2.3	0.826 \pm 0.072	1553 \pm 101	40.4 \pm 0.9	153.2 \pm 8.4	89.5 \pm 2.5	41.7 \pm 0.3	3.66 \pm 0.19
Sebastian	54.3 \pm 1.0	28.1 \pm 1.5	1.742 \pm 0.283	1932 \pm 82	38.7 \pm 1.1	147.0 \pm 5.1	91.6 \pm 1.3	42.6 \pm 0.2	3.79 \pm 0.18
Tipple	53.9 \pm 1.8	20.5 \pm 1.1	1.107 \pm 0.099	1692 \pm 106	39.7 \pm 1.4	168.1 \pm 4.9	91.1 \pm 1.5	42.3 \pm 0.2	3.27 \pm 0.05
Westminster	50.8 \pm 0.9	21.7 \pm 0.5	1.111 \pm 0.190	2033 \pm 291	43.3 \pm 0.6	161.7 \pm 9.9	90.2 \pm 1.1	41.6 \pm 0.5	3.82 \pm 0.16
<i>Capsella</i>	6.2 \pm 1.5	6.8 \pm 1.0	0.132 \pm 0.021	383 \pm 49	52.5 \pm 3.6	94.4 \pm 6.3	90.1 \pm 1.2	35.8 \pm 0.7	5.81 \pm 0.38
<i>Euphorbia</i>	10.2 \pm 0.8	3.5 \pm 0.3	0.051 \pm 0.002	100 \pm 9	46.9 \pm 3.2	208.6 \pm 25.3	77.9 \pm 0.5	43.5 \pm 0.2	4.78 \pm 0.15
<i>Poa</i>	18.0	24.2	0.436	763	58.1	148.6	92.5	37.0	5.07
<i>Senecio</i>	20.7 \pm 2.5	12.9 \pm 2.2	0.477 \pm 0.163	1117 \pm 261	63.9 \pm 4.0	66.1 \pm 1.7	87.9 \pm 0.5	37.7 \pm 1.3	4.35 \pm 0.24
<i>Stellaria</i>	16.5 \pm 2.5	43.3 \pm 5.6	10.383 \pm 2.430	666 \pm 131	62.6 \pm 6.9	85.9 \pm 6.0	91.1 \pm 1.3	37.0 \pm 0.6	3.37 \pm 0.26

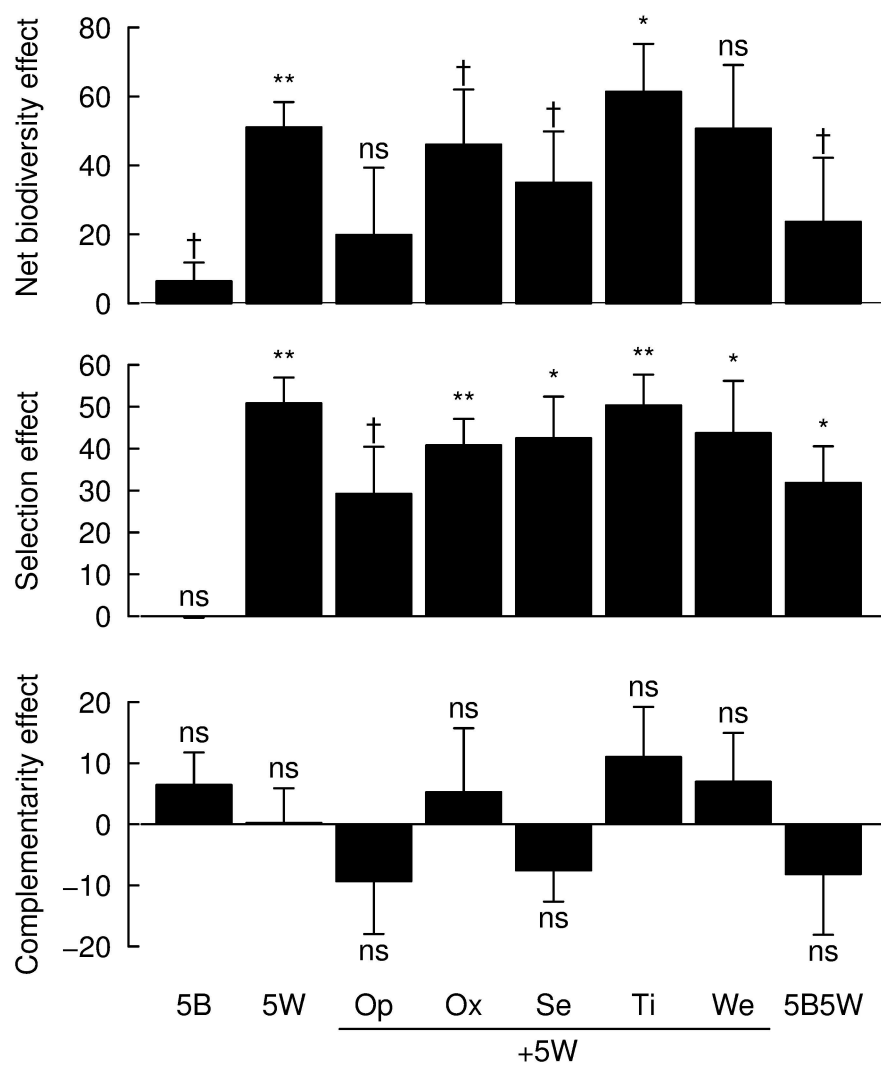
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Fig. 1



754 **Fig. 2**



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757 **Fig. 3**

